Mild hyperbilirubinaemia as an endogenous mitigator of overweight and obesity: Implications for improved metabolic health

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Abstract

Background and aims: Mild endogenous elevation of unconjugated bilirubin (UCB) as seen in Gilbert's syndrome (GS), might mitigate cardiovascular disease (CVD) risk factors including overweight/obesity. This study aimed to determine whether hyperbilirubinaemia is linked to improved anthropometric data and lipid profile.

Methods: Our study considered GS and age-/gender-matched healthy controls (n=248). Additionally, obese female type 2 diabetic patients (DM2) (n=26) were included as a "disease control group".

Results: BMI, hip circumference (HC), and lipid profile were significantly lower in GS. UCB was inversely correlated with BMI ($p<0.001$), HC as well as with fat mass (FM) and lipid variables ($p<0.05$). Moreover, DM2 patients had significantly lower UCB compared to GS and healthy controls. Older GS subjects (≥ 35 years) had significantly reduced anthropometric data and improved lipid profile.

Conclusions: Our results propose that the health promoting potential of mild hyperbilirubinaemia may extend to protection from age-related weight gain and dyslipidaemia.

Key words: Bilirubin, Unconjugated bilirubin (UCB), cardiovascular disease (CVD) risk factors, anthropometric data, overweight/obesity, lipid profile

1- Introduction

Obesity is a life-threatening public health issue and is associated with chronic diseases including type 2 diabetes mellitus (DM2) and cardiovascular disease (CVD) [1]. Risk factors of CVD and premature mortality include unfavourable alterations in body weight, body composition and lipid metabolism [2, 3]. These risk factors, however, seem to be less prevalent in those 5-10% of the general population presenting with Gilbert's syndrome (GS). This hereditary condition is based on various underlying promoter polymorphisms in the UDP glucuronosyltransferase 1A1 (*UGT1A1*) gene (e.g. *UGT1A1*28*) leading to a reduced conjugating activity of this enzyme, phenotypically resulting in a mild increase in unconjugated bilirubin (UCB) (total bilirubin >17.1 $\mu\text{mol/l}$) [4].

Scattered data have shown an inverse association of UCB with weight, body mass index (BMI), and abdominal obesity, suggesting it may play a role in CVD/DM2 risk mitigation, especially in the elderly [5-7]. In that respect, Andersson et al. demonstrated that weight loss (over 4 weeks) was associated with an increase in bilirubin, however, this period was too short to examine cardiovascular risk factors (overweight, hyperlipidaemia or DM2) [8]. Phenotypically, the Framingham heart study showed that higher serum bilirubin was associated with lower risk of CVD [9] and a different study -(meta-analysis, ~15,000 men)-, demonstrated that each 1 $\mu\text{mol/l}$ increase in serum bilirubin was associated with a 6.5% CVD risk reduction [10].

This study aimed to investigate whether (i) hyperbilirubinaemia is correlated to CVD risk factors (anthropometric and lipid data), (ii) these effects are age and gender dependent, and (iii) UCB is associated with metabolic changes in a high risk CVD group of DM2 subjects.

2- Materials and methods

2.1. Data collection

A total of 248 subjects were included in this study. ALT (alanine transferase), AST (aspartate aminotransferase), γ -GT (γ -glutamyl transferase), LDH (lactate dehydrogenase), ALP (alkaline phosphatase), haemoglobin and haematocrit were measured at initial screening examinations. Exclusion criteria for these studies included age <20 yrs, pregnancy, chronic disease, alcoholism (>7 standard drinks/week), smoking (>1 cigarettes/day), excessive physical activity (>10 h/week) and intake of any medication or supplements [11, 12]. Allocation to the GS or healthy control group (HCG) was based on total serum bilirubin (cut-off: 17.1 μ mol/l or=1 mg/dl) [13]. Both groups (n=124 each) were matched for gender and age. Furthermore, the study population was divided into older and younger subsets (cut-off: 35 yrs). In addition to this main study population, female DM2 patients (n=26) were introduced as an age-matched “disease control group” to both older female groups (GS and HCG).

All anthropometric measurements were obtained from participants who were barefooted and lightly dressed in the mornings of the study days. Body height was measured by stadiometer (model 214, Seca) to the nearest 0.5 cm and body weight using standard analogous scales (Selecta 791, Seca). Waist circumference (WC) and hip circumference (HC) were measured by tape (model 203, Seca). BMI and WHR (waist-to-hip ratio) were calculated as using the equations $BMI = kg/m^2$ and $WHR = WC/HC$, respectively. To determine body fat mass (FM), Bioelectric

Impedance Analysis (BIA) was used (BIA Analyser 2000-S, Data-Input GmbH, Germany).

For each subject, an overnight fasting blood sample was collected into serum tubes. Samples were kept cool and protected from light until being analysed or aliquoted (samples aliquots were stored at -80°C for further analyses). Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were analysed using clinical routine diagnostic tests.

Serum UCB was analysed following a well-established high-performance liquid chromatography (HPLC) protocol [12, 13]. Briefly, serum UCB was measured by HPLC (Merck, Hitachi, LaChrom), equipped with a Fortis C18 HPLC column (4.6×150 mm, $3\ \mu\text{m}$), a Phenomenex SecurityGuard™ cartridges for C18 HPLC columns (4×3 mm) and a photodiode array detector (PDA, Shimadzu). All studies had been approved by local ethical committees (274/2010, 1164/2014, 1987/2013) [11, 12, 14] and were conducted in accordance with the Declaration of Helsinki. All participants provided signed informed consent.

2.2. Statistical analysis

Statistical analyses were performed using SPSS (IBM statistics, Version 23.0). Prior to analysis, missing values had been excluded. A $p < 0.05$ was considered significant for all procedures.

The Kolmogorov Smirnov test was used to determine data distribution. For comparison of two groups, the independent samples t -test (parametric data) or Mann-Whitney U -test (non-parametric data) were used. For comparison of three groups, one-way analysis of variance (ANOVA) with Bonferroni adjustment (parametric data), Kruskal-Wallis H -test with pairwise comparisons (non-parametric data) and Chi-square test were performed. Analysis of covariance (ANCOVA) was

performed where needed. Correlation between variables were analysed by Pearson or Spearman correlation. Subsequently, stepwise multiple logistic regression analysis was performed separately for both the younger and the older subgroups, to identify significant predictors for potential dependent variables.

3- Results

3.1. Age-matched GS (Gilbert's syndrome) group the HCG (healthy control group)

The GS group had higher serum UCB (30.7 ± 11.4 vs. 8.7 ± 3.6 , $p < 0.001$), lower BMI (8%, $p < 0.001$) and HC (4%, $p < 0.01$) compared to the HCG. 25% of GS and 46% of the HCG were overweight/obese ($p < 0.01$). Further biologically relevant yet non-significant differences in WC, abdominal circumference (AC), WHR and the FM were found between the groups (Table 1).

Lipid parameters were significantly lower in GS compared to the HCG ($p < 0.05$): 7% LDL; 8% TG; 6% TC; 12% TC/HDL; 15% LDL/HDL (Table 1). For the entire study population ($n = 248$), UCB was inversely correlated with BMI ($r = -0.268$, $p < 0.001$), HC ($r = -0.249$, $p < 0.01$) and FM ($r = -0.226$, $p < 0.05$) and all lipid variables ($p < 0.01$) except HDL.

To back up those bivariate relationships, stepwise linear regression analysis was performed for both age subgroups ($\leq$ 35 yrs, Supplementary Table 3).

In the older group, we found UCB serum concentrations to explain variation in FM (adjusted R square = 0.207, $p = 0.001$) and TG levels (adjusted R square = 0.089, $p = 0.003$). No associations were found in the younger group. In this group, furthermore, no significant differences in anthropometric data (except BMI) and lipid profile were found between GS and the HCG. However, older GS subjects (≥ 35 yrs),

had significantly improved anthropometric and lipid variables (13% BMI, 6% HC, and 26% FM, 15% LDL and TG, 9% TC, 20% TC/HDL, 28% LDL/HDL and 13% greater HDL) relative to their HCG counterparts ($p<0.05$; Table 1). The prevalence of overweight/obesity among older GS (35%) was nearly half that of their controls (69%, $p<0.01$).

3.2. Age-matched females (≥ 35 yrs.) in the GS (Gilbert's syndrome), HCG (healthy control group) and DM2 (type 2 diabetic patients) groups

In addition to GS and the HCG, an older female age-matched disease group with DM2 was included ($n=26$ each group) (Fig. 1). The mean age in GS, HCG and DM2 was 53.2 ± 9.8 | 52.5 ± 10.4 | 54.5 ± 8.6 , and the mean fasting plasma glucose and HbA1C were 4.6 ± 0.5 , 4.7 ± 0.8 | 4.9 ± 0.9 , 4.8 ± 0.9 | 9.04 ± 2.3 , 7.8 ± 0.8 , respectively.

UCB was significantly lower in DM2 compared to the HCG and GS (3.6 ± 2 | 7.2 ± 3 | 25.8 ± 7 $\mu\text{mol/l}$; $p<0.05$), whereas BMI (36.5 ± 9 | 26.9 ± 4 | 23.0 ± 4 kg/m^2 , $p<0.001$), WC (105.0 ± 14 | 86.9 ± 14 | 77.9 ± 11 cm ; $p<0.001$), HC (119.2 ± 16 | 101.9 ± 10 | 96.1 ± 8 cm ; $p<0.001$), and AC (114.1 ± 17 | 90.1 ± 13 | 83.4 ± 9 ; $p<0.001$) were significantly greater. Relative to GS subjects (but not to the HCG), the WHR of DM2 was significantly increased (0.88 ± 0.1 | 0.80 ± 0.1 ; $p=0.003$). The prevalence of overweight/obesity in GS, the HCG and DM2 was 25%, 69% and 100%, respectively ($p<0.001$).

To correct for the intake of statin in 15 DM2 patients, lipid parameters were analysed using ANCOVA with statin-intake as covariate. HDL was significantly lower in DM2 compared to GS ($p<0.001$) and tended to be lower relative to the HCG ($p=0.075$). The HCG had the highest LDL, which was significantly increased compared to GS ($p=0.013$) but not to DM2 (due to the effect of statins). TG were elevated in DM2 compared to the HCG ($p=0.042$) and GS ($p=0.029$). The ratios of TC/HDL and

LDL/HDL were both significantly lower in female GS compared to the HCG and DM2 groups ($p<0.05$; Fig. 1). In addition, highly significant correlations were found between UCB and BMI ($r = -0.646$; $p<0.001$), WC ($r = -0.621$; $p<0.001$), HC ($r = -0.631$; $p<0.001$), AC ($r = -0.532$; $p<0.001$), WHR ($r = -0.402$; $p<0.01$), HDL ($r = 0.518$; $p<0.001$), LDL ($r = -0.334$; $p=0.007$), TG ($r = -0.282$; $p=0.025$), TC/HDL ($r = -0.468$; $p<0.001$) and LDL/HDL ($r = -0.501$; $p<0.001$) when considering subjects from all three groups ($n=78$; controlled for statin-intake; Supplementary Fig. 1).

4- Discussion

The results presented highlight the potential of bilirubin metabolism in contributing to the prevention of overweight/obesity, particularly during aging. In our study, 25% of GS subjects, compared to 46% in the HCG were overweight/obese. In the older subgroup (mean age: 52 yrs), these figures increased to 35% and 69%, respectively. Data analysis revealed significantly lower abdominal obesity in GS compared to the HCG, indicated by a smaller HC and WC, remarkably in the older group. When regarding gender in addition to age, FM was a better predictor of CVD risk factors in women, while central obesity was in men (Supplementary Table 1) [15]. Our results confirm that older male GS had a significantly smaller HC (by approximately 6 cm) and an improved lipid profile (lower TG and ratios of TC/HDL and LDL/HDL), whereas older female GS had a 32% lower FM and lower LDL compared to their control (Supplementary Table 2).

For the purpose of multivariate linear regression analysis (Supplementary Table 3), the study cohort was divided into age subgroups. In the older population (≥ 35 yrs), variation in UCB was significantly explained by the variable FM. In view of the fact that UCB levels are known to decline with increasing age and that FM simultaneously increases in controls (Supplementary Table 2), this result is particularly remarkable.

In accordance with this observation, regression analysis revealed that each 1% increase in FM was associated with an expected decrease of UCB by about 0.8 $\mu\text{mol/l}$ (unstandardised $B = -0.811$, $p < 0.001$). *Vice versa*, FM was expected to drop by roughly 0.3% per each 1 $\mu\text{mol/l}$ incline in UCB (unstandardised $B = -0.272$, $p = 0.001$). These results are in line with what has been reported previously by Andersson et al. [8], assuming that weight loss is typically characterized by a reduction in FM.

It is a generally accepted, well-observed fact that females have higher FM and at the same time lower UCB levels than their male counterparts. Interestingly, variation in the variable FM was significantly explained by the independent variable sex. So, the factor FM might in fact be a key towards explaining not only UCB in general but also differing UCB levels observed between the genders.

As for plasma lipids, which when elevated are accepted risk factors for atherosclerosis [16-18], we demonstrated significantly lower levels in GS vs. HCG. Except for HDL, all other lipid parameters were inversely correlated to UCB [17], the same was found previously by Mölzer et al. [11] and similar results have been reported by Bhuiyan and coworkers [5]. Our stepwise multivariate linear regression analyses revealed that exclusively UCB independently explained variation in TG. In this context, each 1 $\mu\text{mol/l}$ increase in UCB was expected to lower TG by 0.011 mmol/l (unstandardised $B = -0.011$, $p = 0.004$) in the older age group. It has been suggested [11] that UCB through its direct explanatory power for features of body composition and energy turnover (phosphorylated AMPK) and indirectly explained downstream variation in lipid parameters. Similar results were obtained in the present study where (in the older group) UCB substantially accounted for variation in FM, which in turn contributed to explaining markers of the lipid metabolism (TC/HDL and LDL/HDL; Supplementary Table 3).

Generally, those age related effects observed were most impressive, indicating significantly lower lipid parameters (9-28%) and anthropometric measures (6-26%) in GS vs. HCG (≥ 35 years of age), thereby suggesting an extended healthy life-span through protection against age related CVD risk factors. Particularly the LDL/HDL ratio, a valid tool to evaluate the CVD risk and vascular atherosclerotic changes [19], was 28% lower in older GS than in controls. The approximately 9 and 8 cm smaller WC and AC, respectively, further support decreased CVD risk in older GS [16]. In summary, our findings point to a significant role of the UCB metabolism in contributing to the protection of age-related overweight, obesity and unfavorable lipid profile, which seems to be a stronger contributor to CVD protection than the well-known antioxidative effects of bilirubin [10].

In order to further investigate the health promoting potential of circulating bilirubin, a “disease control group” with DM2 that is clearly associated with obesity and metabolic disease was introduced to this study. Compared to GS and the HCG, diabetic patients had significantly reduced UCB concentrations, with unfavorable anthropometric and lipid profiles. All diabetic patients had a BMI ≥ 25 kg/m², whereas 69% of HCG and only 25% of GS were overweight/obese. This supports a hypothesis of an anti-obesogenic and lipid-lowering effect of altered bilirubin metabolism/GS. According to Mendelian randomization (MR) studies, just a few biomarkers were shown to be causally associated with DM2. Out of these, bilirubin reached significant associations with DM2 and related consequences. In one of our previous studies we found that UCB and the *UGT1A1*28* genotype indirectly explained the glucose metabolism, once more through exerting direct upstream effects on energy metabolism, including AMPK and PPAR alpha [11]. In summary, these findings support that higher circulating bilirubin could decrease the risk of DM2 and its complications [20] or delay the onset of DM2 as recently shown [21]. Others

[22, 23] have furthermore suggested a role for bilirubin in protecting against diabetic complications, including the metabolic syndrome and vascular complications. By contrast, the yet largest MR study did not show an association between phenotypical hyperbilirubinaemia and the risk of ischemic heart disease (IHD) [24]. Here it should be noted that, the effects of the (various) GS genotype on phenotypic characteristics other than bilirubin and their consequences on disease outcomes remain inconclusive. It is conceivable that other risk factors for these conditions that are not necessarily related to bilirubin levels such as alcohol intake, smoking or other genetic factors, may play a much stronger role in the etiology of these diseases.

To conclude these intriguing findings, GS individuals are metabolically healthier than age- and gender matched controls, which was demonstrated by a relatively improved body composition and lipid profile, in association with mild hyperbilirubinaemia. This was even more pronounced in older subjects, who are at greater risk of chronic age-related disease. Underlying mechanisms are underexplored but might include a differentially regulated energy metabolism in GS [11], elegantly connecting UCB with glucose- and lipid metabolism and proposing bilirubin as a possible PPAR agonist [25]. With reference to the normo-lipidaemia in generally observed GS, furthermore a role for bilirubin in regulating aryl hydrocarbon receptor (AhR)-dependent cholesterol biosynthesis has been discussed [7].

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

N.S.K. performed statistical evaluation (together with C.M.) and wrote the main part of the manuscript. A.G. included the diabetic group and performed statistics on this part of the dataset. C.M., M.W., D.D., R.M., A.B. and K-H.W. were involved in the main studies which were used for this MS. K-H.W. supervised statistical analyses, drafted the MS together with N.S.K. and provided financial support.

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References

- [1] K. Dhana, M.A. Berghout, A. Peeters, M.A. Ikram, H. Tiemeier, A. Hofman, W. Nusselder, M. Kavousi, O.H. Franco, Obesity in older adults and life expectancy with and without cardiovascular disease, *International journal of obesity* (2005), 40 (2016) 1535-1540.
- [2] G. Joshy, R.J. Korda, J. Attia, B. Liu, A.E. Bauman, E. Banks, Body mass index and incident hospitalisation for cardiovascular disease in 158 546 participants from the 45 and Up Study, *International journal of obesity* (2005), 38 (2014) 848-856.
- [3] C.C. Kee, M.G. Sumarni, K.H. Lim, S. Selvarajah, J. Haniff, G.H. Tee, K. Gurpreet, Y.A. Faudzi, N.M. Amal, Association of BMI with risk of CVD mortality and all-cause mortality, *Public health nutrition*, (2017) 1-9.
- [4] K.H. Wagner, M. Wallner, C. Molzer, S. Gazzin, A.C. Bulmer, C. Tiribelli, L. Vitek, Looking to the horizon: the role of bilirubin in the development and prevention of age-related chronic diseases, *Clinical science* (London, England : 1979), 129 (2015) 1-25.
- [5] A.R. Bhuiyan, S.R. Srinivasan, W. Chen, A. Sultana, G.S. Berenson, Association of serum bilirubin with pulsatile arterial function in asymptomatic young adults: the Bogalusa Heart Study, *Metabolism: clinical and experimental*, 57 (2008) 612-616.

- [6] E.H. Temme, J. Zhang, E.G. Schouten, H. Kesteloot, Serum bilirubin and 10-year mortality risk in a Belgian population, *Cancer causes & control : CCC*, 12 (2001) 887-894.
- [7] A.C. Bulmer, H.J. Verkade, K.H. Wagner, Bilirubin and beyond: a review of lipid status in Gilbert's syndrome and its relevance to cardiovascular disease protection, *Prog Lipid Res*, 52 (2013) 193-205.
- [8] C. Andersson, P. Weeke, E.L. Fosbol, B. Brendorp, L. Kober, W. Coutinho, A.M. Sharma, L. Van Gaal, N. Finer, W.P. James, I.D. Caterson, R.A. Rode, C. Torp-Pedersen, Acute effect of weight loss on levels of total bilirubin in obese, cardiovascular high-risk patients: an analysis from the lead-in period of the Sibutramine Cardiovascular Outcome trial, *Metabolism: clinical and experimental*, 58 (2009) 1109-1115.
- [9] J.P. Lin, C.J. O'Donnell, J.P. Schwaiger, L.A. Cupples, A. Lingenhel, S.C. Hunt, S. Yang, F. Kronenberg, Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham Heart Study, *Circulation*, 114 (2006) 1476-1481.
- [10] L. Novotny, L. Vitek, Inverse relationship between serum bilirubin and atherosclerosis in men: a meta-analysis of published studies, *Experimental biology and medicine (Maywood, N.J.)*, 228 (2003) 568-571.
- [11] C. Molzer, M. Wallner, C. Kern, A. Tosevska, U. Schwarz, R. Zadnikar, D. Doberer, R. Marculescu, K.H. Wagner, Features of an altered AMPK metabolic pathway in Gilbert's Syndrome, and its role in metabolic health, *Scientific reports*, 6 (2016) 30051.
- [12] M. Wallner, A.C. Bulmer, C. Molzer, E. Mullner, R. Marculescu, D. Doberer, M. Wolzt, O.F. Wagner, K.H. Wagner, Haem catabolism: a novel modulator of inflammation in Gilbert's syndrome, *European journal of clinical investigation*, 43 (2013) 912-919.
- [13] M. Wallner, S.M. Blassnigg, K. Marisch, M.T. Pappenheim, E. Mullner, C. Molzer, A. Nersesyan, R. Marculescu, D. Doberer, S. Knasmuller, A.C. Bulmer, K.H. Wagner, Effects of unconjugated bilirubin on chromosomal damage in individuals with Gilbert's syndrome measured with the micronucleus cytome assay, *Mutagenesis*, 27 (2012) 731-735.
- [14] A. Grindel, B. Guggenberger, L. Eichberger, C. Poppelmeyer, M. Gschaidner, A. Tosevska, G. Mare, D. Briskey, H. Brath, K.H. Wagner, Oxidative Stress, DNA Damage and DNA Repair in Female Patients with Diabetes Mellitus Type 2, *PloS one*, 11 (2016) e0162082.
- [15] X. Bi, S.L. Tey, C. Leong, R. Quek, Y.T. Loo, C.J. Henry, Correlation of adiposity indices with cardiovascular disease risk factors in healthy adults of Singapore: a cross-sectional study, *BMC obesity*, 3 (2016) 33.
- [16] A.C. Bulmer, J.T. Blanchfield, I. Toth, R.G. Fassett, J.S. Coombes, Improved resistance to serum oxidation in Gilbert's syndrome: a mechanism for cardiovascular protection, *Atherosclerosis*, 199 (2008) 390-396.
- [17] H.J. Hwang, S.H. Kim, Inverse relationship between fasting direct bilirubin and metabolic syndrome in Korean adults, *Clinica chimica acta; international journal of clinical chemistry*, 411 (2010) 1496-1501.
- [18] G. Lippi, G. Targher, M. Montagnana, G.L. Salvagno, G.C. Guidi, Bilirubin concentration and cardiovascular risk profile, *Liver international : official journal of the International Association for the Study of the Liver*, 29 (2009) 315-316.
- [19] S.K. Kunutsor, F. Zaccardi, J. Karppi, S. Kurl, J.A. Laukkanen, Is High Serum LDL/HDL Cholesterol Ratio an Emerging Risk Factor for Sudden Cardiac Death? Findings from the KIID Study, *Journal of atherosclerosis and thrombosis*, (2016).
- [20] A. Abbasi, Mendelian randomization studies of biomarkers and type 2 diabetes, *Endocrine connections*, 4 (2015) 249-260.
- [21] Y.J. Kwon, Y.J. Lee, B.J. Park, K.W. Hong, D.H. Jung, Total serum bilirubin and 8-year incident type 2 diabetes mellitus: The Korean Genome and Epidemiology Study, *Diabetes & metabolism*, (2017).
- [22] T. Inoguchi, S. Sasaki, K. Kobayashi, R. Takayanagi, T. Yamada, Relationship between Gilbert syndrome and prevalence of vascular complications in patients with diabetes, *Jama*, 298 (2007) 1398-1400.
- [23] J. Nano, T. Muka, M. Cepeda, T. Voortman, K. Dhana, A. Brahimaj, A. Dehghan, O.H. Franco, Association of circulating total bilirubin with the metabolic syndrome and type 2 diabetes: A

systematic review and meta-analysis of observational evidence, *Diabetes & metabolism*, 42 (2016) 389-397.

[24] S. Stender, R. Frikke-Schmidt, B.G. Nordestgaard, P. Grande, A. Tybjaerg-Hansen, Genetically elevated bilirubin and risk of ischaemic heart disease: three Mendelian randomization studies and a meta-analysis, *Journal of internal medicine*, 273 (2013) 59-68.

[25] D.E. Stec, K. John, C.J. Trabbic, A. Luniwal, M.W. Hankins, J. Baum, T.D. Hinds, Jr., Bilirubin Binding to PPARalpha Inhibits Lipid Accumulation, *PloS one*, 11 (2016) e0153427.

Legends to Figures

Fig. 1. Comparison of age-matched females (≥ 35 yrs) in GS, the HCG and DM2 group (n=26, each).

Differences between groups were tested using one-way ANOVA with Bonferroni adjustment for pairwise comparisons. For non-parametric data, Kruskal-Wallis H-test with pairwise comparisons and Bonferroni adjustment were used instead. For lipid parameters, ANCOVA was performed with statin-intake as covariate as 15 DM2 patients reported to be under statin treatment. Significance was assumed with $p < 0.05$. * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$. [†] Indicates a trend with $p < 0.1$.

Supplementary Fig. 1. Correlation analyses of UCB with anthropometric data and lipid metabolism data of older female subjects (n=78; 26 GS subjects, 26 healthy controls, 26 DM2 patients age-matched; age ≥ 35 yrs).

Correlations analyses were performed using the procedure of Spearman. For lipid metabolism, statin intake was included as covariate in the statistical model. Significance was assumed when $p < 0.05$. R-values and p-values are given.

Table 1

Demographic and biochemical parameters of the study population

Parameter	GS	HCG	Correlation to UCB (n=248)	GS	HCG	GS	HCG
	(n=124)	(n=124)		<35 yrs		≥35 yrs	
	(m/f:79/45)	(m/f:79/45)		(n=69)	(n=69)	(n=55)	(n=55)
	(m/f:79/45)	(m/f:79/45)		(m/f:50/19)	(m/f:50/19)	(m/f:29/26)	(m/f:29/26)
Age (yrs)	37.9±14.8	37.6±14.8	-0.072	26.6±3.9	26.6±4.0	52.1±10.7	51.6±11.1
UCB (μmol/l)	30.7±11.4	8.7±3.6 ^a		30.6±11.1	9.6±3.6 ^a	30.7±12.0	7.7±3.3 ^a
BMI (kg/m ²)	23.0±3.2	25.1±4.4 ^a	-0.268 ^a	22.5±3.0	23.5±3.3 ^c	23.8±3.4	27.2±4.6 ^a
NW/OW/O	90/26/4	66/41/16 ^b		56/9/3	49/16/3	34/17/1	17/25/13 ^a
WC (cm)	80.9±11.1	85.8±15.3	-0.144	78.5±10.0	78.7±11.5	83.2±11.7	92.5±15.5 ^b
HC (cm)	96±7.5	100.1±9.5 ^b	-0.249 ^b	95.1±7.6	97.2±7.4	96.9±7.4	103.0±10.4 ^b
WHR	0.8±0.1	0.8±0.1	-0.036	0.8±0.2	0.8±0.1	0.8±0.1	0.9±0.1 ^c
AC (cm)	85.9±10.2	90.7±14.5	-0.113	84.9±10.5	86.2±11.6	86.8±10.1	95.0±15.8 ^c
FM (%)	22±6.4	25.0±8.6	-0.226 ^c	22.2±6.0	21.7±5.9	21.8±7.1	29.3±9.6 ^b
HDL (mmol/l)	1.6±0.4	1.6±0.4	0.044	1.6±0.4	1.6±0.4	1.7±0.5	1.5±0.4 ^c
LDL (mmol/l)	2.7±1.0	2.9±0.9 ^b	-0.239 ^a	2.5±0.9	2.6±0.8	2.9±1.0	3.4±0.8 ^b
TG (mmol/l)	1.1±0.9	1.2±0.7 ^c	-0.223 ^b	1.1±0.7	1.0±0.6	1.2±1.1	1.4±0.6 ^b
TC (mmol/l)	4.8±1.2	5.1±1.0	-0.215 ^b	4.5±1.1	4.7±0.9	5.1±1.2	5.6±0.9 ^c
TC/HDL	3.0±1.0	3.4±1.2 ^b	-0.216 ^b	3.0±0.9	3.0±0.9	3.2±1.2	4.0±1.3 ^a
LDL/HDL	1.7±0.8	2.0±1.0 ^b	-0.220 ^b	1.6±0.7	1.7±0.8	1.8±0.9	2.5±1.0 ^a

GS, Gilbert's syndrome; HCG, healthy control group; yrs., years; m, male; f, female; UCB, unconjugated bilirubin; NW/OW/O, normal weight/overweight/obese; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; FM, fat mass; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol.

Data are expressed as mean value $\pm$ SD. For analysis between two groups, independent *t*-test and Mann-Whitney-*U* test were used. Correlations analyses were performed using the Spearman or Pearson procedure. Significance was assumed with: ^a for $p < 0.001$, ^b for $p < 0.01$, ^c for $p < 0.05$.

Highlights

- Mildly elevated unconjugated bilirubin (UCB) as seen in Gilbert's syndrome (GS) might mitigate cardiovascular disease (CVD) risk factors including overweight/obesity.
- Significantly beneficial body composition and lipid profile were associated in Gilbert's syndrome individuals, so they were metabolically healthier than age- and gender matched controls.
- Health promoting potential of mild hyperbilirubinaemia in the protection from age-related weight gain and dyslipidaemia in Gilbert's syndrome was shown.